



**EFSA ; Scientific Opinion on Flavouring Group Evaluation 94, Revision 1 (FGE.94Rev1): Consideration of aliphatic amines and amides evaluated in an addendum to the group of aliphatic and aromatic amines and amides evaluated by the JECFA (68th meeting)**

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## SCIENTIFIC OPINION

### Scientific Opinion on Flavouring Group Evaluation 94, Revision 1 (FGE.94Rev1):

#### Consideration of aliphatic amines and amides evaluated in an addendum to the group of aliphatic and aromatic amines and amides evaluated by the JECFA (68<sup>th</sup> meeting)<sup>1</sup>

#### EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)<sup>2,3</sup>

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#### ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to consider evaluations of flavouring substances assessed since 2000 by the Joint FAO/WHO Expert Committee on Food Additives (the JECFA), and to decide whether further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. The present consideration concerns a group of 12 aliphatic amines and amides evaluated by the JECFA at the 68<sup>th</sup> meeting in 2007. This revision of the consideration is made due to additional toxicity data available for two substances, *N*-3,7-dimethyl-2,6-octadienyl cyclopropylcarboxamide [FL-no: 16.095] and *N*-[(ethoxycarbonyl)methyl]-*p*-menthane-3-carboxamide [FL-no: 16.111]. The substances were evaluated through a stepwise approach that integrates information on structure-activity relationships, intake from current uses, toxicological threshold of concern and available data on metabolism and toxicity. The Panel agrees with the application of the Procedure as performed by the JECFA for 11 of the substances considered in this FGE and agrees with the JECFA conclusion, “No safety concern at estimated levels of intake as flavouring substances” based on the MSDI

<sup>1</sup> On request from the European Commission, Question No EFSA-Q-2011-01120 and EFSA-Q-2012-00079, adopted on 24 May 2012.

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approach. For one substance [FL-no: 16.090] additional toxicity data are still needed before the evaluation can be finalised. Besides the safety assessment of these flavouring substances, the specifications for the materials of commerce have also been considered and for one substance, [FL-no: 16.090], the composition of the stereoisomeric mixture has to be specified.

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**KEY WORDS**

JECFA 68<sup>th</sup> meeting, food safety, flavourings, aliphatic amines, aliphatic amides.

## SUMMARY

The European Food Safety Authority (EFSA) asked the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) to provide scientific advice to the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was requested to consider the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000. These flavouring substances are listed in the Register, which was adopted by Commission Decision 1999/217/EC and its consecutive amendments.

The consideration deals with 12 aliphatic amines and amides [FL-no: 16.090, 16.095, 16.098, 16.099, 16.100, 16.101, 16.102, 16.103, 16.104, 16.105, 16.111 and 17.035], which are in the Register and which were evaluated by the JECFA at its 68<sup>th</sup> meeting.

The present revision is made due to additional toxicity data requested in the previous opinion have been provided for *N*-3,7-dimethyl-2,6-octadienyl cyclopropylcarboxamide [FL-no: 16.095] and *N*-(ethoxycarbonyl)methyl]-*p*-menthane-3-carboxamide [FL-no: 16.111].

The Panel concluded that no supporting Flavouring Group Evaluation was available for the substances in the present FGE.

Genotoxicity data from *in vitro* and *in vivo* studies were available for seven [FL-no: 16.090, 16.095, 16.098, 16.099, 16.102, 16.103 and 16.111] of the flavouring substances and the results did not indicate any concern for genotoxicity of the substances in this flavouring group.

In the previous version of FGE.94 the Panel concluded that it could agree with the way the application of the Procedure has been performed by the JECFA for nine substances. For three substances [FL-no: 16.090, 16.095 and 16.111] no adequate NOAEL were available. New 90-day studies have now become available for [FL-no: 16.095 and 16.111] and NOAELs to provide adequate margins of safety can be derived.

For 3-(3,4-Dimethoxyphenyl)-*N*-[2-(3,4-dimethoxyphenyl)-ethyl]-acrylamide [FL-no: 16.090] the JECFA used a NOAEL from a 90-day study in rats for *N*-nonanoyl-4-hydroxy-3-methoxybenzylamide ([FL-no: 16.006] considered in FGE.86) to provide an adequate margin of safety. However, the Panel considers this substance not sufficiently structurally related to [FL-no: 16.090] to be used as a supporting substance.

Thus, for one substance [FL-no: 16.090] the Panel concluded that additional toxicity data are still needed before it can be evaluated as a flavouring substance.

For 10 of the 12 substances, use levels have been provided by the Industry. Based on the use levels the mTAMDI figures calculated for nine substances [FL-no: 16.090, 16.095, 16.098, 16.099, 16.102, 16.103, 16.104, 16.111 and 17.035] are above the threshold of concern for their structural classes. For these nine substances more reliable data are needed. On the basis of such data the flavouring substances should be reconsidered using the Procedure. For the remaining two substances [FL-no: 16.100 and 16.101], use levels are needed to calculate the mTAMDI in order to identify those flavouring substances that need more refined exposure assessment.

In order to determine whether the conclusion for the 12 JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications are available for 11 substances [FL-no: 16.095, 16.098, 16.099, 16.100, 16.101, 16.102, 16.103, 16.104, 16.105, 16.111 and 17.035] of the 12 materials of commerce. For one substance [FL-no: 16.090] information on the stereoisomerism has not been specified sufficiently.

Thus, for one of the substance [FL-no: 16.090] the Panel has reservations (additional toxicity data are needed and the composition of the stereoisomeric mixture has to be specified).

For the remaining 11 substances [FL-no: 16.095, 16.098, 16.099, 16.100, 16.101, 16.102, 16.103, 16.104, 16.105, 16.111 and 17.035] the Panel agrees with the JECFA conclusion “No safety concern at estimated levels of intake as flavouring substances” based on the MSDI approach.

## TABLE OF CONTENTS

Abstract .....	1
Summary .....	3
Background .....	6
Terms of Reference as provided by the Commission.....	6
Assessment .....	6
History of the Evaluation of the Substances in the present FGE .....	8
1. Presentation of the Substances in the JECFA Flavouring Group .....	8
1.1. Description.....	8
1.1.1. JECFA Status.....	8
1.1.2. EFSA Considerations .....	8
1.2. Isomers.....	9
1.2.1. Status .....	9
1.2.2. EFSA Considerations .....	9
1.3. Specifications.....	9
1.3.1. JECFA Status.....	9
1.3.2. EFSA Considerations .....	9
2. Intake Estimations .....	9
2.1. JECFA Status.....	9
2.2. EFSA Considerations.....	9
3. Genotoxicity Data.....	10
3.1. Genotoxicity Studies – Text Taken from the JECFA (JECFA, 2008b).....	10
3.2. New Genotoxicity Study on [FL-no: 16.111] .....	12
3.3. EFSA Considerations.....	13
4. <i>In Vitro</i> Hydrolysis Study on [FL-no: 16.111] .....	13
5. 90-Day Studies on [FL-no:16.095 and 16.111].....	14
5.1. 90-day dietary toxicity study in CrI:CD (SD) rats on <i>N</i> -3,7-dimethyl-2,6-octadienyl-cyclopropylcarboxamide [FL-no: 16.095] .....	14
5.2. 90-day oral (by gavage) toxicity study in CrI:CD (SD) rats on <i>N</i> -[(ethoxycarbonyl)methyl]-p-menthane-3-carboxamide [FL-no: 16.111].....	14
6. Application of the Procedure.....	15
6.1. Application of the Procedure to Aliphatic Amines and Amides Substances by the JECFA (JECFA, 2008b).....	15
6.2. EFSA Considerations.....	16
7. Conclusion.....	17
Table 1: Specification Summary .....	19
Table 2: Genotoxicity Data .....	21
Table 3: Summary of Safety Evaluations.....	24
References .....	26
Abbreviations .....	29

## BACKGROUND

Regulation (EC) No 2232/96 of the European Parliament and the Council (EC, 1996a) lays down a Procedure for the establishment of a list of flavouring substances, the use of which will be authorised to the exclusion of all other substances in the EU. In application of that Regulation, a Register of flavouring substances used in or on foodstuffs in the Member States was adopted by Commission Decision 1999/217/EC (EC, 1999a), as last amended by Commission Decision 2009/163/EC (EC, 2009a). Each flavouring substance is attributed a FLAVIS-number (FL-number) and all substances are divided into 34 chemical groups. Substances within a group should have some metabolic and biological behaviour in common.

Substances which are listed in the Register are to be evaluated according to the evaluation programme laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a), which is broadly based on the Opinion of the Scientific Committee on Food (SCF, 1999a).

Commission Regulation (EC) No 1565/2000 lays down that substances that are contained in the Register and will be classified in the future by the Joint FAO/WHO Expert Committee on Food Additives (the JECFA) so as to present no safety concern at current levels of intake will be considered by the European Food Safety Authority (EFSA), who may then decide that no further evaluation is necessary.

In the period 2000 - 2008, during its 55<sup>th</sup>, 57<sup>th</sup>, 59<sup>th</sup>, 61<sup>st</sup>, 63<sup>rd</sup>, 65<sup>th</sup>, 68<sup>th</sup> and 69<sup>th</sup> meetings, the JECFA evaluated about 1000 substances, which are in the EU Register.

## TERMS OF REFERENCE AS PROVIDED BY THE COMMISSION

The European Food Safety Authority (EFSA) is requested to consider the JECFA evaluations of flavouring substances assessed since 2000, and to decide whether no further evaluation is necessary, as laid down in Commission Regulation (EC) No 1565/2000 (EC, 2000a). These flavouring substances are listed in the Register which was adopted by Commission Decision 1999/217 EC (EC, 1999a) and its consecutive amendments.

The evaluation programme was finalised at the end of 2009.

After the finalisation of the evaluation programme, in their letters of the 6<sup>th</sup> October 2011 and 23<sup>rd</sup> December 2011, the Commission requested EFSA to carry out re-evaluation of the flavouring substances, *N*-3,7-dimethyl-2,6-octadienyl cyclopropylcarboxamide [FL-no: 16.095] and *N*-(ethoxycarbonyl)methyl]-*p*-menthane-3-carboxamide [FL-no: 16.111] based on additionally submitted data on toxicity, and depending on the outcome, to proceed to the evaluation of these flavouring substances through the Procedure, also according to Commission Regulation (EC) No 1565/2000 (EC, 2000a).

## ASSESSMENT

The approach used by EFSA for safety evaluation of flavouring substances is referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000a), hereafter named the “EFSA Procedure”. This Procedure is based on the Opinion of the Scientific Committee on Food (SCF, 1999a), which has been derived from the evaluation procedure developed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1995; JECFA, 1996a; JECFA, 1997a; JECFA, 1999b), hereafter named the “JECFA Procedure”. The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) compares the JECFA evaluation of structurally related substances with the result of a corresponding EFSA evaluation, focussing on specifications, intake estimations and toxicity data, especially genotoxicity data. The evaluations by EFSA will conclude whether the flavouring substances are of no safety concern at their estimated levels of intake, whether additional data are required or whether certain substances should not be put through the EFSA Procedure.

The following issues are of special importance.

### *Intake*

In its evaluation, the Panel as a default uses the “Maximised Survey-derived Daily Intake” (MSDI) approach to estimate the *per capita* intakes of the flavouring substances in Europe.

In its evaluation, the JECFA includes intake estimates based on the MSDI approach derived from both European and USA production figures. The highest of the two MSDI figures is used in the evaluation by the JECFA. It is noted that in several cases, only the MSDI figures from the USA were available, meaning that certain flavouring substances have been evaluated by the JECFA only on the basis of these figures. For Register substances for which this is the case the Panel will need EU production figures in order to finalise the evaluation.

When the Panel examined the information provided by the European Flavour Industry on the use levels in various foods, it appeared obvious that the MSDI approach in a number of cases would grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach. It is noted that the JECFA at its 65<sup>th</sup> meeting considered “how to improve the identification and assessment of flavouring agents, for which the MSDI estimates may be substantially lower than the dietary exposures that would be estimated from the anticipated average use levels in foods” (JECFA, 2006c).

In the absence of more accurate information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel has decided also to perform an estimate of the daily intakes per person using a “modified Theoretical Added Maximum Daily Intake” (mTAMDI) approach based on the normal use levels reported by Industry.

As information on use levels for the flavouring substances has not been requested by the JECFA or has not otherwise been provided to the Panel, it is not possible to estimate the daily intakes using the mTAMDI approach for the substances evaluated by the JECFA. The Panel will need information on use levels in order to finalise the evaluation.

### *Threshold of 1.5 Microgram/Person/Day (Step B5) Used by the JECFA*

The JECFA uses the threshold of concern of 1.5 microgram/person/day as part of the evaluation procedure:

“The Committee noted that this value was based on a risk analysis of known carcinogens which involved several conservative assumptions. The use of this value was supported by additional information on developmental toxicity, neurotoxicity and immunotoxicity. In the judgement of the Committee, flavouring substances for which insufficient data are available for them to be evaluated using earlier steps in the Procedure, but for which the intake would not exceed 1.5 microgram per person per day would not be expected to present a safety concern. The Committee recommended that the Procedure for the Safety Evaluation of Flavouring Agents used at the forty-sixth meeting be amended to include the last step on the right-hand side of the original procedure (“Do the condition of use result in an intake greater than 1.5 microgram per day?”) (JECFA, 1999b).

In line with the Opinion expressed by the Scientific Committee on Food (SCF, 1999), the Panel does not make use of this threshold of 1.5 microgram per person per day.

### *Genotoxicity*

As reflected in the Opinion of SCF (SCF, 1999a), the Panel has in its evaluation focussed on a possible genotoxic potential of the flavouring substances or of structurally related substances.



Generally, substances for which the Panel has concluded that there is an indication of genotoxic potential *in vitro*, will not be evaluated using the EFSA Procedure until further genotoxicity data are provided. Substances for which a genotoxic potential *in vivo* has been concluded, will not be evaluated through the Procedure.

### *Specifications*

Regarding specifications, the evaluation by the Panel could lead to a different opinion than that of JECFA, since the Panel requests information on e.g. isomerism.

### *Structural Relationship*

In the consideration of the JECFA evaluated substances, the Panel will examine the structural relationship and metabolism features of the substances within the flavouring group and compare this with the corresponding FGE.

## **HISTORY OF THE EVALUATION OF THE SUBSTANCES IN THE PRESENT FGE**

In FGE.94, which contains a group of 12 aliphatic amines and amides, the Panel considered that additional toxicity data were needed for three substances [FL-no: 16.090, 16.095 and 16.111] before they could be evaluated as flavouring substances, as no adequate toxicity study from which a no observed adverse effect level (NOAEL) could be established was available, neither on the substances nor on supporting substances.

<b>FGE</b>	<b>Opinion adopted by EFSA</b>	<b>Link</b>	<b>No. of candidate substances</b>
FGE.94	23 September 2009	<a href="http://www.efsa.europa.eu/en/scdocs/scdoc/1338.htm">http://www.efsa.europa.eu/en/scdocs/scdoc/1338.htm</a>	12
FGE.94Rev1	24 May 2012		12

Additional toxicity data have now been provided for two substances [FL-no: 16.095 and 16.111]. The data provided are 90-day studies for both substances. Furthermore, new metabolism and genotoxicity data have been provided for [FL-no: 16.111].

Since the publication of FGE.94, information on the stereoisomeric composition has been provided by ECHA for three substances [FL-no: 16.090, 16.102 and 16.104] and ID tests and solubility in ethanol have been provided for two substances [FL-no: 16.100 and 16.101] (ECHA, 2010a).

## **1. Presentation of the Substances in the JECFA Flavouring Group**

### **1.1. Description**

#### **1.1.1. JECFA Status**

The JECFA has evaluated a group of 12 flavouring substances consisting of aliphatic and aromatic amines and amides at the 68<sup>th</sup> meeting (JECFA, 2007c).

#### **1.1.2. EFSA Considerations**

All of the JECFA evaluated substances are in the Register. This consideration therefore deals with these 12 substances. The Panel concluded that there are no supporting substances from other FGEs for the aliphatic amines and amides evaluated by the JECFA (JECFA, 2007c).

## **1.2. Isomers**

### **1.2.1. Status**

The following five substances [FL-no: 16.102, 16.103, 16.104, 16.105 and 16.111] have one or more chiral centres. Two substances can exist as geometrical isomers [FL-no: 16.090 and 16.095].

### **1.2.2. EFSA Considerations**

For one substance [FL-no: 16.090] the composition of the stereoisomeric mixture has to be specified. Adequate information on isomeric composition is available for the remaining substances.

For the three stereoisomeric substances [FL-no: 16.095, 16.105 and 16.111], the CAS register number (CASrn) is considered to specify the stereoisomeric composition (Table 1).

## **1.3. Specifications**

### **1.3.1. JECFA Status**

The JECFA specifications are available for all substances (JECFA, 2008c).

### **1.3.2. EFSA Considerations**

The European Flavour Industry has submitted specifications for all 12 substances commercially used in Europe (Flavour Industry, 2007h; Flavour Industry, 2008b; Flavour Industry, 2006u; Flavour Industry, 2006v; Flavour Industry, 2004l; Flavour Industry, 2006z; EFFA, 2006x). Although the JECFA specifications are available, the specifications used in this consideration are those submitted by the Industry. See Table 1.

Specifications including complete purity criteria and identity are available for 11 substances [FL-no: 16.095, 16.098, 16.099, 16.100, 16.101, 16.102, 16.103, 16.104, 16.105, 16.111 and 17.035].

The stereoisomeric composition has not been specified sufficiently for one substance [FL-no: 16.090] (see Section 1.2.2 and Table 1).

## **2. Intake Estimations**

### **2.1. JECFA Status**

For all 12 substances evaluated by the JECFA intake data are available for the EU.

### **2.2. EFSA Considerations**

For 10 JECFA evaluated substances [FL-no: 16.090, 16.095, 16.098, 16.099, 16.102, 16.103, 16.104, 16.105, 16.111 and 17.035] normal and maximum use levels have been provided by the Flavour Industry in accordance with the Commission Regulation (EC) No 1565/2000 (Flavour Industry, 2007h; Flavour Industry, 2008b; Flavour Industry, 2006u; Flavour Industry, 2006v; Flavour Industry, 2004l; Flavour Industry, 2006z; EFFA, 2006x; EC, 2000a) (see Table 2.2.1). Based on the normal use levels, mTAMDI figures (see Table 2.2.2) can be calculated (for calculation of mTAMDI figures, see e.g. FGE.03, Annex II (EFSA, 2004d)).

**Table 2.2.1 Normal and Maximum use levels (mg/kg) available for JECFA evaluated substances in FGE.94**

FL-no	Food Categories																	
	Normal use levels (mg/kg)																	
	Maximum use levels (mg/kg)																	
	01.0	02.0	03.0	04.1	04.2	05.0	06.0	07.0	08.0	09.0	10.0	11.0	12.0	13.0	14.1	14.2	15.0	16.0
16.090	3	-	5	-	-	10	-	10	-	-	-	-	5	-	3	5	10	5
	10	-	10	-	-	20	-	50	-	-	-	-	20	-	20	25	30	10
16.095	0,01	0,01	0,1	-	-	1	-	0,1	0,01	0,01	-	-	0,01	-	1	1	0,01	0,01
	0,05	0,05	0,5	-	-	5	-	0,5	0,05	0,05	-	-	0,05	-	5	5	0,05	0,05
16.098	1	2	-	1	1	-	-	1	1	1	-	-	5	-	-	-	5	1
	3	4	-	3	3	-	-	2	3	3	-	-	10	-	-	-	10	3
16.099	1	2	-	1	1	-	-	1	1	1	-	-	5	-	-	-	5	1
	3	4	-	3	3	-	-	2	3	3	-	-	10	-	-	-	10	3
16.102	50	50	50	-	-	50	50	50	50	50	50	50	50	50	50	50	50	50
	200	200	200	-	-	200	200	200	200	200	200	200	200	200	200	200	200	200
16.103	50	50	50	-	-	50	50	50	50	50	50	50	50	50	50	50	50	50
	200	200	200	-	-	200	200	200	200	200	200	200	200	200	200	200	200	200
16.104	5	-	5	5	-	5	-	-	-	-	-	-	5	-	5	5	5	-
	15	-	15	15	-	15	-	-	-	-	-	-	15	-	15	15	15	-
16.105	-	5	-	-	-	-	-	-	5	-	-	-	5	-	-	-	5	-
	-	15	-	-	-	-	-	-	15	-	-	-	15	-	-	-	15	-
16.111	10	10	10	10	10	10	10	20	10	10	-	-	-	20	10	10	50	10
	300	200	150	200	200	200	50	200	100	100	-	-	-	300	50	400	350	200
17.035	30	30	20	-	-	30	30	50	20	-	-	-	-	30	30	40	20	30
	100	100	100	-	-	100	100	300	200	-	-	-	-	200	200	300	100	100

**Table 2.2.2 Estimated intakes based on the MSDI- and the mTAMDI approach**

FL-no	EU Register name	MSDI – EU (µg/capita/day)	MSDI – USA (µg/capita/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
16.102	2,3,4,5,6-Pentahydroxy-N-(2-hydroxyethyl)-hexanamide	24	13	27000	Class I	1800
16.103	(2R)-2-Hydroxy-N-(2-hydroxyethyl)propanamide	24	10	27000	Class I	1800
16.104	2-[(2-Hydroxypropanoyl)amino]ethyl dihydrogen phosphate	12	5	2700	Class I	1800
16.105	2-[(2,3,4,5,6-Pentahydroxyhexanoyl)amino]ethyl dihydrogen phosphate	12	3	870	Class I	1800
17.035	4-Amino-butyric acid	0.12	0.1	18000	Class I	1800
16.090	3-(3,4-Dimethoxy-phenyl)-N-[2-(3,4-dimethoxyphenyl)-ethyl]-acrylamide	1.2	1	3000	Class III	90
16.095	N-3,7-Dimethyl-2,6-octadienyl cyclopropylcarboxamide	61	31	380	Class III	90
16.098	N-(1-Propylbutyl)-1,3-benzodioxole-5-carboxamide	0.012	0.1	470	Class III	90
16.099	N-(2,4-Dimethoxy-benzyl)-N'-(2-pyridin-2-yl-ethyl)-oxalamide	0.012	0.2	470	Class III	90
16.100	N1-(2-Methoxy-4-methylbenzyl)-N2-(2-(5-methylpyridin-2-yl)ethyl)oxalamide	0.012	0.01	ND	Class III	90
16.101	N1-(2-Methoxy-4-methylbenzyl)-N2-(2-(pyridin-2-yl)ethyl)oxalamide	0.012	0.01	ND	Class III	90
16.111	N-[(Ethoxycarbonyl)methyl]-p-menthane-3-carboxamide	37	34	7400	Class III	90

ND: No intake data available

### 3. Genotoxicity Data

#### 3.1. Genotoxicity Studies – Text Taken<sup>4</sup> from the JECFA (JECFA, 2008b)

*In vitro* and *in vivo* genotoxicity testing has been performed on six flavouring substances [FL-no: 16.090, 16.098, 16.099, 16.102, 16.103 and 16.111] in this group. The results of these studies are summarized in Table 2.1 and described below.

<sup>4</sup> The text is taken verbatim from the indicated reference source, but text related to substances not included in the present FGE has been removed.

### *In Vitro*

*N*-(Heptan-4-yl)benzo[*d*][1,3]dioxole-5-carboxamide ([FL-no: 16.098] *N*-(1-propylbutyl)-1,3-benzodioxole-5-carboxamide), *N*-gluconyl ethanolamine ([FL-no: 16.102] 2,3,4,5,6-pentahydroxy-*N*-(2-hydroxyethyl)-hexanamide), *N*-lactoyl ethanolamine ([FL-no: 16.103] (2*R*)-2-hydroxy-*N*-(2-hydroxyethyl)propanamide) and *N*-[2-(3,4-dimethoxyphenyl)ethyl]-3,4-dimethoxycinnamic acid amide [FL-no: 16.090] 3-(3,4-dimethoxyphenyl)-*N*-[2-(3,4-dimethoxyphenyl)-ethyl]-acrylamide) were tested in *Salmonella typhimurium* TA98, TA100, TA102, TA1535 and TA1537 and *Escherichia coli* WP2uvrA at concentrations up to 5000 µg/plate, with and without S9 activation. There was no evidence of an increase in revertants (Uhde, 2004b; Verspeek-Rip, 2004a; Verspeek-Rip, 2004b; Zhang, 2004a).

*N*1-(2,4-Dimethoxybenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)-oxalamide ([FL-no: 16.099] *N*-(2,4-dimethoxybenzyl)-*N'*-(2-pyridin-2-yl-ethyl)-oxalamide) induced an increase in the number of revertants in *S. typhimurium* TA1535 in the absence (but not in the presence) of metabolic activation compared with control values; however, no dose-response was observed, and the mean number of revertants was reported to be below historical spontaneous reversion or negative control values. When tested under the conditions of the preincubation assay at concentrations of up to 5000 µg/plate, *N*1-(2,4-dimethoxybenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)-oxalamide [FL-no: 16.099] induced an increase in the number of revertants in *S. typhimurium* TA100 in the presence of metabolic activation, but only at a concentration of 62 µg/plate; no dose-response pattern was observed, and no significant increases in the number of revertants were reported in the absence of metabolic activation at concentrations of up to 5000 µg/plate. Moreover, *N*1-(2,4-dimethoxybenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)-oxalamide [FL-no: 16.099] consistently tested negative in several other strains of *S. typhimurium* (TA98 and TA1537) and in *E. coli* WP2uvrA in both the absence and presence of metabolic activation, in both plate incorporation and preincubation assays, at concentrations of up to 5000 µg/plate. Given the lack of a dose-dependent response, non-reproducibility of results and the fact that the number of revertants was below historical control values, it was concluded that *N*1-(2,4-dimethoxybenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)-oxalamide [FL-no: 16.099] was non-mutagenic (Zhang, 2005a).

*N*-[(Ethoxycarbonyl)methyl]-*p*-menthane-3-carboxamide [FL-no: 16.111] induced a slight increase in the number of revertants in *S. typhimurium* TA100 and TA1535 in the absence of metabolic activation compared with control values; however, the increase was not statistically significant. In a set of confirmatory experiments, *S. typhimurium* TA100 and TA1535 were retested at concentrations of 0, 2000, 3000, 4000 or 5000 µg/plate without metabolic activation. The study reported an increase in revertant colonies in strain TA1535 that was reproducible and, at the highest concentration tested, was significantly above in-house historical controls. The report concluded that *N*-[(ethoxycarbonyl)methyl]-*p*-menthane-3-carboxamide [FL-no: 16.111] was weakly mutagenic to TA1535 under the test conditions. In contrast, increases observed in the revertant colonies in *S. typhimurium* TA100, although statistically significant, were small and did not follow a dose-response pattern. Moreover, *N*-[(ethoxycarbonyl)methyl]-*p*-menthane-3-carboxamide [FL-no: 16.111] consistently tested negative in several other strains of *S. typhimurium* (TA98 and TA1537) and in *E. coli* WP2uvrA in both the absence and presence of metabolic activation, at concentrations of up to 5000 µg/plate (Thompson, 2005).

*N*-(Heptan-4-yl)benzo[*d*][1,3]dioxole-5-carboxamide [FL-no: 16.098] and *N*1-(2,4-dimethoxybenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)-oxalamide [FL-no: 16.099] produced no evidence of genotoxicity at 0, 21, 62, 190, 560, 1670 or 5000 µg/ml in standard chromosomal aberration assays in Chinese hamster ovary cells cultured with and without S9 metabolic activation (Zhang, 2004b; Zhang, 2005b).

### *In Vivo*

In a standard mouse micronucleus bone marrow assay, groups of 21 male Swiss albino (CD-1) mice per dose were injected intraperitoneally with 0, 175, 350 or 700 mg *N*-(heptan-4-yl)benzo[*d*][1,3]dioxole-5-carboxamide [FL-no: 16.098]/kg bw. At 24, 36 and 48 hours following

dose administration, seven mice from each group were killed, and their femoral bone marrow was harvested, fixed and stained. No statistically significant differences were observed in the number of polychromatic erythrocytes with micronuclei between the test groups and the negative control (Pucaj, 2004a).

In a standard mouse micronucleus bone marrow assay using the same protocol as described above, groups of 21 male Swiss albino (CD-1) mice per dose were injected intraperitoneally with 0, 200, 400 or 800 mg *N*1-(2,4-dimethoxybenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)oxalamide [FL-no: 16.099]/kg bw. At 24, 36 and 48 hours following dose administration, seven mice from each group were killed, and their femoral bone marrow was harvested, fixed and stained. No statistically significant differences were observed in the number of polychromatic erythrocytes with micronuclei between the test groups and the negative control (Pucaj, 2004b).

In a similar standard mouse micronucleus bone marrow assay, male NMRI BR mice (five per group) were administered aqueous *N*-gluconyl ethanolamine [FL-no: 16.102] at 0 (negative or positive control) or 2000 mg/kg bw via gavage. Femoral bone marrow was isolated at 24 or 48 hours post-administration. Treatment and control mice showed no difference in the ratio of polychromatic to normochromatic erythrocytes. *N*-Gluconyl ethanolamine [FL-no: 16.102] showed no mutagenic potential in the mouse micronucleus assay (Buskens, 2004a).

Employing the same standard mouse micronucleus bone marrow assay as used above, male NMRI BR mice (five per group) were administered aqueous *N*-lactoyl ethanolamine [FL-no: 16.103] at 0 (negative or positive control) or 2000 mg/kg bw via gavage. Femoral bone marrow was isolated 24 or 48 hours after administration. Treated and control mice showed no difference in the ratio of polychromatic to normochromatic erythrocytes. *N*-Lactoyl ethanolamine [FL-no: 16.103] showed no mutagenic potential in the mouse micronucleus assay (Buskens, 2004a).

#### *Conclusion on Genotoxicity*

On the weight of evidence, negative results were obtained with the flavouring agents of this group when tested in *in vitro* mutation assays in *S. typhimurium* and *E. coli*, as well as in mammalian cells. Negative results were also obtained in *in vivo* micronucleus assays.

For a summary of *in vitro* / *in vivo* genotoxicity data considered by the ECFA, see Table 2.1.

### **3.2. New Genotoxicity Study on [FL-no: 16.111]**

A Mouse Lymphoma Assay for [FL-no: 16.111] (Flanders, 2006) was submitted after the publication of FGE.94.

The study was conducted according to an adequate design to assess the potential mutagenicity of the test material on the thymidine kinase, TK +/-, locus of the L5178Y mouse lymphoma cell line (Flanders, 2006). L5178Y TK +/- 3.7.2c mouse lymphoma cells (heterozygous at the thymidine kinase locus) were treated with the test material at eight dose levels, in duplicate, together with vehicle (solvent) and positive controls. The entire experiment was repeated to confirm the result of the first experiment. Four hours exposures were used both with and without activation in Experiment 1. In Experiment 2, the exposure time without activation was increased to 24 hours. The dose range of test material, plated for expression of mutant colonies, was selected following the results of a preliminary cytotoxicity test and was 42.03 to 672.5 µg/ml in the absence of metabolic activation and 84.06 to 1008.75 µg/ml in the presence of metabolic activation for the first experiment. For the second experiment the dose range plated for expression of mutant colonies was 10.51 to 504.38 µg/ml without metabolic activation and 42.03 to 672.5 µg/ml with metabolic activation.

The maximum dose level used was limited by test material induced cytotoxicity. A precipitate of test material was observed at 1345 µg /ml during the course of the study. The vehicle (solvent) controls

had mutant frequency values that were considered acceptable for the L5178Y cell line at the TK +/- locus. The positive control materials induced marked increases in the mutant frequency indicating the satisfactory performance of the test and of the activity of the metabolising system.

The test material did not induce any statistically significant or dose-related increases in the mutant frequency at any dose level, either with or without metabolic activation, in either the first or the second experiment incorporating dose levels that exhibited optimum levels of cytotoxicity.

Study results are presented in Table 2.2.

### 3.3. EFSA Considerations

Genotoxicity data from *in vitro* studies are available for seven substances [FL-no: 16.090, 16.095, 16.098, 16.099, 16.102, 16.103 and 16.111] and *in vivo* studies were available for four substances [FL-no: 16.098, 16.099, 16.102 and 16.103] of the 12 flavouring substances evaluated by the JECFA.

The Panel noted that conflicting positive results were obtained for N-(2,4-dimethoxy-benzyl)-N'-(2-pyridin-2-yl-ethyl)-oxalamide [FL-no: 16.099] in the one study by (Zhang, 2005a) when tested in the *S. typhimurim* TA1535 and TA100. However, no dose-related response was observed, the positive results with TA1535 and TA100 were not reproducible and concomitantly, the tests with TA98 and TA1537 were consistently negative (JECFA, 2008b). The Panel concluded that the available data did not raise concern about genotoxicity in the Ames test.

For the consideration in FGE.94 on N-3,7-dimethyl-2,6-octadienyl cyclopropylcarboxamide [FL-no: 16.095] an additional genotoxicity study was provided by the Industry (Next Century Incorporated, 2004) after the JECFA evaluated the substance at the 68<sup>th</sup> meeting (JECFA, 2007c). In this study, the substance was tested in a bacterial reverse mutation test using *S. typhimurium* strains TA97a, TA98, TA100, TA1535, and *E. coli* strain WP2uvra with and without metabolic activation. It was concluded to be negative for the induction of mutagenicity (see Table 2.3).

N-[(Ethoxycarbonyl)methyl]-p-menthane-3-carboxamide [FL-no: 16.111] did not induce gene mutations at the thymidine kinase locus in L5178Y cells in the Mouse Lymphoma Assay (Flanders, 2006).

Overall, the Panel considered the available data not to raise concern with respect to genotoxicity.

### 4. In Vitro Hydrolysis Study on [FL-no: 16.111]

The hydrolysis of N-[(ethoxycarbonyl)methyl]-p-menthane-3-carboxamide [FL-no: 16.111] was studied in artificial pancreatic juice and rat liver homogenate (Poet et al., 2005). Based on the disappearance of the employed substrate, [FL-no: 16.111] was hydrolysed in artificial pancreatic juice with a half-life of  $43 \pm 14.7$  min. and a first order loss rate (K) of  $1.06 \pm 0.426$  hr<sup>-1</sup>. In 20 fold-diluted liver homogenate the disappearance of [FL-no: 16.111] was considerably faster (half-life:  $0.802 \pm 0.191$  min.). However, the potential hydrolysis products p-menthane-3-carboxylic acid, glycine ethylester and glycine were only detected at trace levels. This indicates that the disappearance of [FL-no: 16.111] under the employed *in vitro*-conditions is due to the hydrolysis of the ethyl ester bond rather than the hydrolysis of the amide bond.

This stability of the amide bond is in agreement with data provided for N-benzonitrile-p-menthan-3-carboxamide, [FL-no: 16.117]. This structurally related substance was not hydrolysed when incubated with pooled hepatic microsomes from male rats or male humans under conditions in which hydrolytic enzymes were shown to be active (Sipes and Kong, 2012).

In conclusion, the Panel considers that the candidate substance [FL-no: 16.111] cannot be expected to be metabolized to innocuous products.



## 5. 90-Day Studies on [FL-no:16.095 and 16.111]

90-day studies requested in the previous version of this FGE were submitted for [FL-no: 16.095 and 16.111] by the Industry.

### 5.1. 90-day dietary toxicity study in Crl:CD (SD) rats on *N*-3,7-dimethyl-2,6-octadienyl-cyclopropylcarboxamide [FL-no: 16.095]

A 90-day dietary toxicity study followed by a 28-day recovery period was performed with *N*-3,7-dimethyl-2,6-octadienyl cyclopropylcarboxamide [FL-no: 16.095] in rats (Bauter, 2011). The study was performed according to OECD guideline (TG 408) under GLP. The dose levels tested were 0, 11, 110, and 1100 mg *N*-3,7-dimethyl-2,6-octadienyl cyclopropylcarboxamide/kg diet, equal to mean daily exposures of 0, 0.7, 7.3, and 73.3 mg/kg bw/day in the male rats and of 0, 0.8, 8.1, and 80.1 mg/kg bw/day in the female rats. Each test group consisted of 10 animals per sex. Recovery groups were included for the control and high dose groups as well. Clinical observations, functional observation battery and motor activity were recorded. The feed homogeneity was checked by dietary chemical analysis. Data on body weight and individual food consumption were collected throughout the in-life phase of the study. Blood samples were taken for complete haematological, clinical chemical and serological analyses. At study termination body weight, organ and tissue weights were recorded after macroscopical examination and complete histopathology was performed on the animals of the control and highest dose groups. No substance related effects were found implying that the highest dose group is the NOAEL of 1100 mg/kg diet which is equal to approximately 73 mg *N*-3,7-dimethyl-2,6-octadienyl cyclopropylcarboxamide /kg bw/day in the male rats.

### 5.2. 90-day oral (by gavage) toxicity study in Crl:CD (SD) rats on *N*-[(ethoxycarbonyl)methyl]-*p*-menthane-3-carboxamide [FL-no: 16.111]

A 90-day oral toxicity study by gavage in Crl:CD (SD) rats followed by 14-day recovery period was performed with *N*-[(ethoxycarbonyl)methyl]-*p*-menthane-3-carboxamide [FL-no: 16.111] (Kirkpatrick, 2011). The study was performed according to OECD guideline (TG 408) under GLP. The substance was administered at doses of 0, 25, 75, 225 and 675 mg *N*-[(ethoxycarbonyl)methyl]-*p*-menthane-3-carboxamide/kg bw/day to 10 animals/sex/dose group via gavage. Recovery groups were included for the control and high dose groups. The following parameters were evaluated: daily clinical observation, weekly recording of individual body weight and food consumption while clinical chemistry, hematology, blood clotting parameters and urinalysis were done at study termination and at the end of the recovery period. A modified Irwin test, performed before start and on week 12 of the study, was conducted in order to evaluate any potential effect on the central nervous system. Ophthalmology examination was performed on week 1 and week 12 of the study. Complete necropsies were conducted on all animals and selected organs were weighted. Selected tissues were examined microscopically from all animals in the control and the 675 mg/kg bw/day groups at the primary necropsy. Kidney, liver, heart and gross lesions were examined microscopically from all animals at the scheduled necropsies. Sections of kidneys were also evaluated for presence of  $\alpha$ -2-globulin by immunohistochemistry. Spermatogenic endpoints (motility, morphology and numbers) were evaluated for all males at the scheduled necropsies.

Test substance related higher neutrophil (in males and females; m+f), monocyte (m), and white blood cell count (m+f) were observed in the high-dose group. These increases declined after the recovery period. Lower hematocrit values were seen at 225 and 675 mg/kg bw/day in the males but not in the females. However, lower hematocrit, accompanied by decrease in hemoglobin, red blood cell count, mean corpuscular volume and increase in red cell distribution width were observed after the recovery period in both sexes at 675 mg/kg/ bw/day.

Higher serum creatinine (m+f), urea nitrogen (m+f), calcium (m), and triglycerides (m+f), and lower serum albumin/globulin ratios (m+f) and chloride levels (m+f) and higher total urine volume and

lower specific gravity were observed at 675 mg/kg bw/day in males and females. These changes were not present after the recovery period.

At gross macroscopy, enlarged kidneys with rough surface were observed in one male and pale kidney was reported for one female rat at 675 mg/kg bw/day, which correlated with the histopathological observation of renal tubular degeneration. An increase in liver and kidney weight was observed in male and female rats at 675 mg/kg bw/day though this finding was no longer present after the recovery period.

Test substance-related microscopic findings were noted in the kidney (tubular degeneration, and dilatation, interstitial fibrosis and tubular epithelium vacuolation) and the liver (periportal hepatocellular vacuolation and centrilobular hepatocellular hypertrophy) in both male and female rats and heart (increase in incidence of cardiomyopathy; females only) at 675 mg/kg bw/day. Furthermore, tubular hyaline droplets were observed in male kidney at all doses. However, this finding was not dose related and considered to correlate with the increase in male rat specific  $\alpha$ -2 $\mu$ -globulin observed at immunohistochemical investigation. Microscopic changes in the kidney, liver and heart also present after the recovery period though they were reduced in severity.

Renal changes observed in the male and female rats and cardiomyopathy in the heart in female rats only at 675 mg/kg bw/day were considered adverse. Haematological changes observed at 225 and 675 mg/kg bw/day were also considered adverse as they were dose related and red cells changes were also present after recovery period of 14 days at the dose of 675 mg/kg bw/day. Therefore the Panel established a NOAEL of 75 mg/kg bw/day.

## 6. Application of the Procedure

### 6.1. Application of the Procedure to Aliphatic Amines and Amides Substances by the JECFA (JECFA, 2008b)

According to the JECFA five of the substances belong to structural class I and seven to structural class III using the decision tree approach presented by Cramer *et al.* (Cramer *et al.*, 1978).

The JECFA concluded five aliphatic amines and amides substances [FL-no: 16.102, 16.103, 16.104, 16.105 and 17.035] at step A3 in the JECFA Procedure – meaning that the substances are expected to be metabolised to innocuous products (step 2) and concluded that the intakes for all substances are below the thresholds for their structural class I (step A3).

The remaining seven flavouring substances in this group cannot be predicted to be metabolized to innocuous products. The estimated daily per capita intakes of these flavouring substances are below the threshold of concern (i.e. 90  $\mu$ g/person per day) for structural class III, and a No Observed Adverse Effect Level (NOAEL) exists to provide an adequate margin of safety to the estimated intake as flavouring substances (step B4).

#### *Step B4.*

For *N*-(1-propylbutyl)-1,3-benzodioxole-5-carboxamide [FL-no: 16.098] (*N*-(heptan-4-yl)benzo[d][1,3]dioxole-5-carboxamide), the no-observed-effect level (NOEL) of 20 mg/kg bw per day from a 93-day study in rats (Kot, 2005a) provides an adequate margin of safety (> 10 million) in relation to the currently estimated level of exposure from its use as a flavouring agent in Europe (0.0002  $\mu$ g/kg body weight (bw) per day) and in the USA (0.002  $\mu$ g/kg bw per day).

For *N*-(2,4-dimethoxy-benzyl)-*N'*-(2-pyridin-2-yl-ethyl)-oxalamide [FL-no: 16.099] (*N*1-(2,4-dimethoxybenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)-oxalamide), the NOEL of 100 mg/kg bw per day from a 93-day study in rats (Kot, 2005b) provides an adequate margin of safety (> 33 million) in relation to the currently estimated level of exposure from its use as a flavouring agent in Europe (0.0002  $\mu$ g/kg



bw per day) and in the USA (0.003 µg/kg bw per day). This NOEL is appropriate for the structurally related flavouring agents *N*1-(2-methoxy-4-methylbenzyl)-*N*2-(2-(5-methylpyridin-2-yl)ethyl)oxalamide [FL-no: 16.100] and *N*1-(2-methoxy-4-methylbenzyl)-*N*2-(2-(pyridin-2-yl)ethyl)oxalamide [FL-no: 16.101], because they are also oxalamides and are expected to be metabolized by similar pathways. For these structurally related flavouring agents, the NOEL of 100 mg/kg bw per day provides an adequate margin of safety (500 million) in relation to the currently estimated levels of exposure to these flavouring agents in both Europe and the USA (0.0002 µg/kg bw per day).

For *N*-[(ethoxycarbonyl)methyl]-*p*-menthane-3-carboxamide [FL-no: 16.111], the NOEL of 8 mg/kg bw per day for the structurally related substance *N*-ethyl 2-isopropyl-5-methylcyclohexane carboxamide ([FL-no: 16.013] considered in FGE.86) from a 28-day study in rats (Miyata, 1995) provides an adequate margin of safety (> 13,000) in relation to the currently estimated level of exposure from its use as a flavouring substance in Europe (0.6 µg/kg bw/day).

For 3-(3,4-dimethoxyphenyl)-*N*-[2-(3,4-dimethoxyphenyl)-ethyl]-acrylamide [FL-no: 16.090] (*N*-[2-(3,4-dimethoxyphenyl)ethyl]-3,4-dimethoxycinnamic acid amide), the NOEL of 8.36 mg/kg bw per day for the structurally related *N*-nonanoyl-4-hydroxy-3-methoxybenzylamide ([FL-no: 16.006] considered in FGE.86) from a 90-day study in rats (Posternak et al., 1969) provides an adequate margin of safety (> 400,000) in relation to the currently estimated level of exposure from its use as a flavouring agent in the USA (0.02 µg/kg bw per day).

For *N*-3,7-dimethyl-2,6-octadienylcyclopropylcarboxamide [FL-no: 16.095], the NOEL of 92 mg/kg bw per day from a 28-day study in rats (Merkel, 2005) provides an adequate margin of safety (> 180,000) in relation to the currently estimated level of exposure from its use as a flavouring agent in the USA (0.5 µg/kg bw per day).

The evaluations of the 12 substances are summarised in Table 3.1: Summary of Safety Evaluation of Aliphatic Amines and Amides (JECFA, 2008b).

## 6.2. EFSA Considerations

In the previous version of FGE.94, the Panel agreed with the way the application of the Procedure has been performed by the JECFA for nine of the 12 aliphatic and aromatic amines and amides. For the other three substances [FL-no: 16.090, 16.095 and 16.111] the Panel did not agree and concluded that additional toxicity data are needed before they can be evaluated as a flavouring substance.

Additional toxicity data have now become available for two substances [FL-no: 16.095 and 16.111]:

Based on the new data submitted (Bauter, 2011) for *N*-3,7-dimethyl-2,6-octadienylcyclopropylcarboxamide [FL-no: 16.095] a NOAEL of 73.3 mg/kg bw/day could be established. When comparing this NOAEL at step B4 in the Procedure to the estimated exposure based on the MSDI (61 microgram *per capita* per day, corresponding to 1 microgram /kg bw/day) an adequate margin of safety of  $7 \times 10^4$  can be calculated.

Based on the new data submitted (Kirkpatrick, 2011) for *N*-[(ethoxycarbonyl)methyl]-*p*-menthane-3-carboxamide [FL-no: 16.111] a NOAEL of 75 mg/kg bw/day could be established. When comparing this NOAEL at step B4 in the Procedure to the estimated exposure based on the MSDI (37 microgram *per capita* per day, corresponding to 0.6 microgram /kg bw/day) an adequate margin of safety of  $12 \times 10^4$  can be calculated.

For 3-(3,4-dimethoxy-phenyl)-*N*-[2-(3,4-dimethoxyphenyl)-ethyl]-acrylamide [FL-no: 16.090] a NOAEL of 8.36 mg/kg bw per day for *N*-nonanoyl-4-hydroxy-3-methoxybenzylamide ([FL-no: 16.006] considered in FGE.86) from a 90-day study in rats (Posternak et al., 1969) was used by the JECFA to provide an adequate margin of safety (> 400,000). However, the Panel considers that *N*-

nonanoyl-4-hydroxy-3-methoxybenzylamide is not sufficiently structurally related to 3-(3,4-dimethoxy-phenyl)-*N*-[2-(3,4-dimethoxyphenyl)-ethyl]-acrylamide [FL-no: 16.090] to be used as a supporting substance.

Thus the Panel concludes that 11 substances can be considered to be of no safety concern at their estimated dietary intake based on the MSDI approach.

For the remaining substance [FL-no: 16.090], additional toxicity data are needed before it can be evaluated as a flavouring substance.

## 7. Conclusion

The JECFA has evaluated a group of 12 flavouring substances consisting of aliphatic amines and amides at the 68<sup>th</sup> meeting. All the JECFA evaluated substances are in the Register, and this consideration therefore deals with these 12 substances [FL-no: 16.090, 16.095, 16.098, 16.099, 16.100, 16.101, 16.102, 16.103, 16.104, 16.105, 16.111 and 17.035].

The Panel concluded that no supporting FGE was available for the substances in the present FGE.

Genotoxicity data from *in vitro* and *in vivo* studies were available for seven [FL-no: 16.090, 16.095, 16.098, 16.099, 16.102, 16.103 and 16.111] of the 12 flavouring substances evaluated by the JECFA and the results did not indicate any concern for genotoxicity of the substances in this flavouring group.

In the previous version of FGE.94 the Panel agreed with the way the application of the Procedure has been performed by the JECFA for nine of 12 substances, but for three substances [FL-no: 16.090, 16.095 and 16.095] no adequate NOAEL were available. Since then 90-day studies have become available for [FL-no: 16.095 and 16.111] and NOAELs to provide adequate margins of safety are derived.

For 3-(3,4-dimethoxyphenyl)-*N*-[2-(3,4-dimethoxyphenyl)-ethyl]-acrylamide [FL-no: 16.090] the JECFA used a NOAEL from a 90-day study in rats for *N*-nonanoyl-4-hydroxy-3-methoxybenzylamide ([FL-no: 16.006] considered in FGE.86) to provide an adequate margin of safety. However, the Panel considers this substance not sufficiently structurally related to [FL-no: 16.090] to be used as a supporting substance.

Accordingly, additional toxicity data are needed before [FL-no: 16.090] can be evaluated as a flavouring substance.

For 10 of the 12 substances, use levels have been provided by the Industry. Based on these use levels the mTAMDI figures calculated for nine substances [FL-no: 16.090, 16.095, 16.098, 16.099, 16.102, 16.103, 16.104, 16.111 and 17.035] are above the threshold of concern for their structural classes. For these substances more reliable data are needed. On the basis of such data the flavouring substances should be reconsidered using the Procedure. Following this procedure additional toxicological data might become necessary. For the remaining two [FL-no: 16.100 and 16.101] of the 12 substances, use levels are needed to calculate the mTAMDI in order to identify those flavouring substances that need more refined exposure assessment.

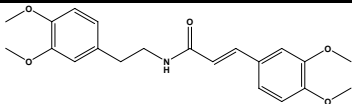
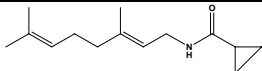
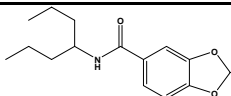
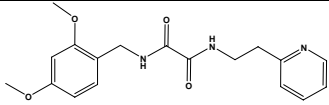
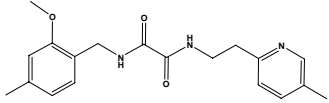
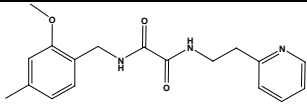
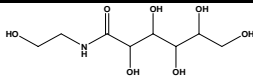
In order to determine whether the conclusion for the JECFA evaluated substances can be applied to the materials of commerce, it is necessary to consider the available specifications. Adequate specifications are available for 11 substances [FL-no: 16.095, 16.098, 16.099, 16.100, 16.101, 16.102, 16.103, 16.104, 16.105, 16.111 and 17.035]. For one substance [FL-no: 16.090] information on the stereoisomerism has not been specified sufficiently.

Thus, for one substance [FL-no: 16.090] the Panel has reservations (additional toxicity data are needed and the composition of the stereoisomeric mixture has to be specified).

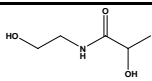
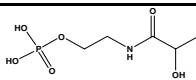
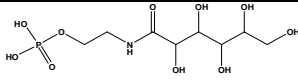
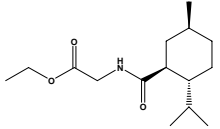
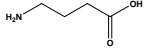
For the remaining 11 substances [FL-no: 16.095, 16.098, 16.099, 16.100, 16.101, 16.102, 16.103, 16.104, 16.105, 16.111 and 17.035] the Panel agrees with JECFA conclusion “No safety concern at estimated levels of intake as flavouring substances” based on the MSDI approach.

**TABLE 1: SPECIFICATION SUMMARY**

**Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Aliphatic and Aromatic Amines and Amides (JECFA, 2008c)**

FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments / References for specifications
16.090 1777	3-(3,4-Dimethoxy-phenyl)-N-[2-(3,4-dimethoxyphenyl)-ethyl]-acrylamide		4310 69444-90-2	Solid C <sub>21</sub> H <sub>25</sub> NO <sub>5</sub> 371.43	Practically insoluble or insoluble Slightly soluble	127.9 IR NMR MS 99 %	n.a. n.a.	Mixture of (Z)- and (E)-isomer (EFFA, 2010a). (Flavour Industry, 2006v). Composition of stereoisomeric mixture to be specified. Register name to be changed to 3-(3,4-Dimethoxyphenyl)-N-[2-(3,4-dimethoxyphenyl)-ethyl]-acrylamide
16.095 1779	N-3,7-Dimethyl-2,6-octadienyl cyclopropylcarboxamide		4267 744251-93-2	Solid C <sub>14</sub> H <sub>23</sub> NO 221.00	Insoluble Soluble	146 (at 1 hPa) 53 IR NMR 98 %	n.a. n.a.	(Flavour Industry, 2004l). CASm refers to (E) isomer. Register name to be changed accordingly.
16.098 1767	N-(1-Propylbutyl)-1,3-benzodioxole-5-carboxamide		4232 745047-51-2	Solid C <sub>13</sub> H <sub>21</sub> NO <sub>3</sub> 263.34	Insoluble Sparingly soluble	116 IR NMR MS 99%	n.a. n.a.	(Flavour Industry, 2006z).
16.099 1768	N-(2,4-Dimethoxy-benzyl)-N'-(2-pyridin-2-yl-ethyl)-oxalamide		4233 745047-53-4	Solid C <sub>18</sub> H <sub>21</sub> N <sub>3</sub> O <sub>4</sub> 343.38	Insoluble Sparingly soluble	123 IR NMR MS 99%	n.a. n.a.	(Flavour Industry, 2006z). [FL-no: 16.099, 16.100 and 16.101] should be named by the same nomenclature principles.
16.100 1769	N1-(2-Methoxy-4-methylbenzyl)-N2-(2-(5-methylpyridin-2-yl)ethyl)oxalamide		4234 745047-94-3	Solid C <sub>19</sub> H <sub>23</sub> N <sub>3</sub> O <sub>3</sub> 341.41	Insoluble Sparingly soluble	- 132-133 IR NMR MS 99%	n.a. n.a.	(Flavour Industry, 2008b). [FL-no: 16.099, 16.100 and 16.101] should be named by the same nomenclature principles.
16.101 1770	N1-(2-Methoxy-4-methylbenzyl)-N2-(2-(pyridin-2-yl)ethyl)oxalamide		4231 745047-97-6	Solid C <sub>18</sub> H <sub>21</sub> N <sub>3</sub> O <sub>3</sub> 327.38	Insoluble Sparingly soluble	- 128-129 IR NMR 99%	n.a. n.a.	(Flavour Industry, 2008b). [FL-no: 16.099, 16.100 and 16.101] should be named by the same nomenclature principles.
16.102 1772	2,3,4,5,6-Pentahydroxy-N-(2-hydroxyethyl)-hexanamide		4254 686298-93-1	Solid C <sub>8</sub> H <sub>19</sub> NO <sub>7</sub> 239.22	Soluble Soluble	99-100 IR NMR MS 99%	n.a. 1.562	Mixture of diastereoisomers (EFFA, 2010a). Only one diastereomer (2R,3S,4S,5R) (Flavour Industry, 2012b). Register name to be changed to

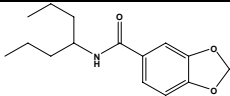
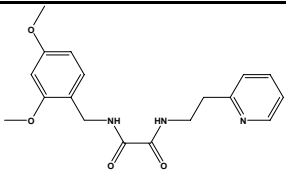
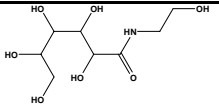
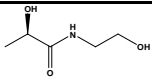
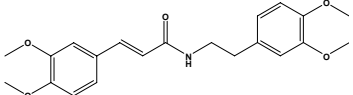
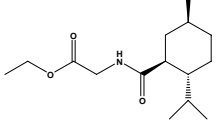
**Table 1: Specification Summary of the Substances in the JECFA Flavouring Group of Aliphatic and Aromatic Amines and Amides (JECFA, 2008c)**

FL-no JECFA-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Phys.form Mol.formula Mol.weight	Solubility 1) Solubility in ethanol 2)	Boiling point, °C 3) Melting point, °C ID test Assay minimum	Refrac. Index 4) Spec.gravity 5)	EFSA comments / References for specifications
16.103 1774	(2R)-2-Hydroxy-N-(2-hydroxyethyl)propanamide		4256 5422-34-4	Liquid $C_5H_{11}NO_3$ 133.15	Soluble Moderate	380 IR NMR MS 99%	1.481-1.491 1.185-1.196	(2R,3S,4S,5R)-2,3,4,5,6-Pentahydroxy-N-(2-hydroxyethyl)hexanamide. (Flavour Industry, 2008b).  (Flavour Industry, 2008b). CASm refers to the racemate. Register name or CASm to be changed accordingly.
16.104 1775	2-[(2-Hydroxypropanoyl)amino]ethyl dihydrogen phosphate		782498-03-7	Solid $C_5H_{12}NO_6$ 213.13	Soluble Moderate	200 IR NMR MS 95%	n.a. 1.521	(Flavour Industry, 2008b; EFSA, 2006x). Racemate (EFSA, 2010a).
16.105 1773	2-[(2,3,4,5,6-Pentahydroxyhexanoyl)amino]ethyl dihydrogen phosphate		4255 791807-20-0	Solid $C_8H_{18}NO_{10}P$ 319.21	Soluble Moderate	130 IR NMR MS 95%	n.a. 1.76	(Flavour Industry, 2008b; EFSA, 2006x). CASm refers to the (2R,3S,4S,5R) isomer. Register name to be changed accordingly.
16.111 1776	N-[(Ethoxycarbonyl)methyl]-p-menthane-3-carboxamide		4309 68489-14-5	Solid $C_{15}H_{27}NO_3$ 269.38	Practically insoluble Soluble	151 (2.7 hPa) 80-82 IR NMR MS 99%	n.a. n.a.	(Flavour Industry, 2007h). CASm refers to the (1R,2S,5R) isomer. Register name to be changed accordingly.
17.035 1771	4-Amino-butyric acid		56-12-2	Solid $C_4H_9NO_2$ 103.12	Slightly soluble Practically insoluble	200 IR NMR MS 100 %	n.a. n.a.	(Flavour Industry, 2006u).

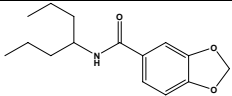
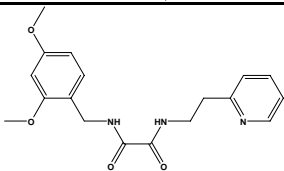
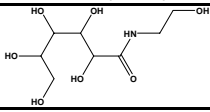
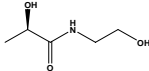
- 1) Solubility in water, if not otherwise stated.
- 2) Solubility in 95% ethanol, if not otherwise stated.
- 3) At 1013.25 hPa, if not otherwise stated.
- 4) At 20°C, if not otherwise stated.
- 5) At 25°C, if not otherwise stated.

**TABLE 2: GENOTOXICITY DATA**

**Table 2.1: Summary of Genotoxicity Data of Aliphatic and Aromatic Amines and Amides Evaluated by the JECFA (JECFA, 2008b)**

FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
<b><i>In vitro</i></b>							
16.098 1767	N-(1-Propylbutyl)-1,3-benzodioxole-5-carboxamide		Reverse mutation <sup>a</sup>	<i>S. typhimurium</i> TA98, TA100, TA1535 and TA1537	0, 21, 62, 190, 560, 1670 or 5000 µg/plate <sup>b</sup>	Negative <sup>c</sup>	(Zhang, 2004a)
			Reverse mutation <sup>a</sup>	<i>E. coli</i> WP2uvrA	0, 21, 62, 190, 560, 1670 or 5000 µg/plate <sup>b</sup>	Negative <sup>c</sup>	(Zhang, 2004a)
			Chromosomal aberration	Chinese hamster ovary cells	0, 21, 62, 190, 560, 1670 or 5000 µg/ml	Negative <sup>c</sup>	(Zhang, 2004b)
16.099 1768	N-(2,4-Dimethoxy-benzyl)-N'-(2-pyridin-2-yl-ethyl)-oxalamide		Reverse mutation <sup>a</sup>	<i>S. typhimurium</i> TA98, TA100, TA1535 and TA1537	0, 21, 62, 190, 560 or 1670 µg/plate	Weakly positive/negative <sup>c,d</sup>	(Zhang, 2005a)
			Reverse mutation <sup>a</sup>	<i>E. coli</i> WP2uvrA	0, 21, 62, 190, 560, 1670 or 5000 µg/plate	Negative <sup>c</sup>	(Zhang, 2005a)
			Chromosomal aberration	Chinese hamster ovary cells	0, 21, 62, 190, 560, 1670 or 5000 µg/plate	Negative <sup>c</sup>	(Zhang, 2005b)
16.102 1772	2,3,4,5,6-Pentahydroxy-N-(2-hydroxyethyl)-hexanamide		Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535 and TA1537	0, 3, 10, 33, 100, 333, 1000, 3330 or 5000 µg/plate	Negative <sup>c</sup>	(Verspeek-Rip, 2004a)
			Reverse mutation	<i>E. coli</i> WP2uvrA	0, 3, 10, 33, 100, 333, 1000, 3330 or 5000 µg/plate	Negative <sup>c</sup>	(Verspeek-Rip, 2004a)
16.103 1774	(2R)-2-Hydroxy-N-(2-hydroxyethyl)propanamide		Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535 and TA1537	0, 3, 10, 33, 94, 310, 940, 3140 or 4720 µg/plate	Negative <sup>c</sup>	(Verspeek-Rip, 2004b)
			Reverse mutation	<i>E. coli</i> WP2uvrA	0, 3, 10, 33, 94, 310, 940, 3140 or 4720 µg/plate	Negative <sup>c</sup>	(Verspeek-Rip, 2004b)
16.090 1777	3-(3,4-Dimethoxy-phenyl)-N-[2-(3,4-dimethoxyphenyl)-ethyl]-acrylamide		Reverse mutation <sup>a</sup>	<i>S. typhimurium</i> TA98, TA100, TA102, TA1535 and TA1537	0, 31.6, 100, 316, 1000 or 3160 µg/plate	Negative <sup>c</sup>	(Uhde, 2004b)
16.111 1776	N-[(Ethoxycarbonyl)methyl]-p-menthane-3-carboxamide		Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535 and TA1537	0, 50, 150, 500, 1500, 2000 <sup>f</sup> , 3000 <sup>f</sup> , 4000 <sup>f</sup> or 5000 µg/plate	Weakly positive <sup>c,g</sup>	(Thompson, 2005)
			Reverse mutation	<i>E. coli</i> WP2uvrA	0, 50, 150, 500, 1500 or 5000 µg/plate	Negative <sup>c</sup>	(Thompson, 2005)

**Table 2.1: Summary of Genotoxicity Data of Aliphatic and Aromatic Amines and Amides Evaluted by the JECFA (JECFA, 2008b)**

FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
<b><i>In vivo</i></b>							
16.098 1767	N-(1-Propylbutyl)-1,3-benzodioxole-5-carboxamide		Micronucleus induction	Swiss albino (CD-1) mice	0, 175, 350 or 700 mg/kg bw <sup>h</sup>	Negative	(Pucj, 2004a)
16.099 1768	N-(2,4-Dimethoxy-benzyl)-N'-(2-pyridin-2-yl-ethyl)-oxalamide		Micronucleus induction	Swiss albino (CD-1) mice	0, 200, 400 or 800 mg/kg bw <sup>h</sup>	Negative	(Pucj, 2004b)
16.102 1772	2,3,4,5,6-Pentahydroxy-N-(2-hydroxyethyl)-hexanamide		Micronucleus induction	NMRI BR mice	0 or 2000 mg/kg bw <sup>i</sup>	Negative	(Buskens, 2004a)
16.103 1774	(2R)-2-Hydroxy-N-(2-hydroxyethyl)propanamide		Micronucleus induction	NMRI BR mice	0 or 2000 mg/kg bw <sup>i</sup>	Negative	(Buskens, 2004a)

<sup>a</sup> Plate incorporation assay and preincubation assay.

<sup>b</sup> The maximum concentration tested was 1670 µg/plate except for *Salmonella typhimurium* TA100 in the plate incorporation assay, for *S. typhimurium* TA98 and TA100 and *Escherichia coli* WP2uvrA in the preincubation assay without S9 (9000 × g supernatant from rat liver) and for *S. typhimurium* TA98, TA1535 and TA1537 and *E. Coli* WP2uvrA in the preincubation assay with S9, because of precipitation.

<sup>c</sup> With and without metabolic activation.

<sup>d</sup> In the plate incorporation assay, *S. typhimurium* TA1535 tested positive at concentrations of 21, 190 and 1670 µg/plate, but only without S9. In the preincubation assay, *S. typhimurium* TA100 tested positive only at 62 µg/plate and only with S9.

<sup>e</sup> For *S. typhimurium* TA100 only.

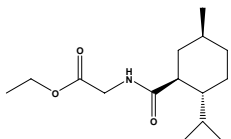
<sup>f</sup> *S. typhimurium* TA100 and TA1535 tested without S9 using both plate incorporation and preincubation methods.

<sup>g</sup> Weak incidence of reverse mutation observed in *S. typhimurium* TA100 and TA1535. All other strains showed no evidence of mutagenicity.

<sup>h</sup> Test material administered via single intraperitoneal injection.

<sup>i</sup> Test material administered via single gavage dose.

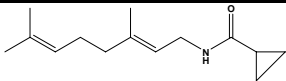
**Table 2.2: Summary of Additional Genotoxicity Data on *N*-[(ethoxycarbonyl)methyl]-*p*-menthane-3-carboxamide Submitted by Industry**

FL-no JECFA-no	EU Register name JECFA name	Structural formula	Test System	Test Object	Route	Dose	Reported Result	Reference
16.111 1776	<i>N</i> -[(ethoxycarbonyl)methyl]- <i>p</i> -menthane-3-carboxamide		Mouse Lymphoma	L5178Ytk+/- mouse lymphoma cells	Oral	42.03 to 672.5 micrograms/ml	Negative <sup>1</sup>	(Flanders, 2006)
						84.06 to 1008.75 micrograms/ml	Negative <sup>2</sup>	
			Mouse Lymphoma	L5178Ytk+/- mouse lymphoma cells	Gavage	10.51 to 504.38 micrograms/ml	Negative <sup>1</sup>	(Flanders, 2006)
						42.03 to 672.5 micrograms/ml	Negative <sup>2</sup>	

<sup>1</sup> Without metabolic activation.

<sup>2</sup> With metabolic activation.

**Table 2.3: Summary of Additional Genotoxicity Data on *N*-3,7-dimethyl-2,6-octadienyl cyclopropylcarboxamide Submitted by Industry**

FL-no JECFA-no	EU Register name JECFA name	Structural formula	End-point	Test system	Concentration	Results	Reference
16.095 1779	<i>N</i> -3,7-dimethyl-2,6-octadienyl cyclopropylcarboxamide		Reverse mutation	<i>S. typhimurium</i> TA97a, TA98, TA100, TA1535	0, 5, 10, 50, 100, 500, 1000, 2000, 2500 or 5000 µg/plate	Negative <sup>1</sup>	(Next Century Incorporated, 2004)
			Reverse mutation	<i>E. coli</i> WP2uvrA	0, 50, 100, 500, 1000 or 2000 µg/plate	Negative <sup>1</sup>	(Next Century Incorporated, 2004)

<sup>1</sup> With and without metabolic activation.

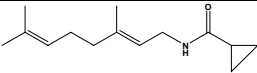
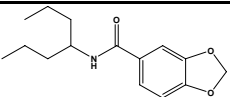
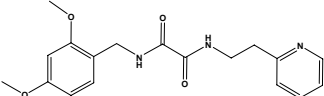
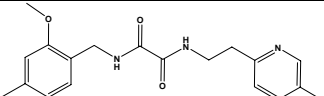
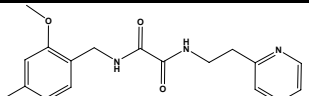
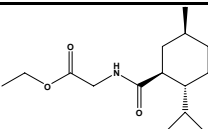


**TABLE 3: SUMMARY OF SAFETY EVALUATIONS**

**Table 3.1: Summary of Safety Evaluation of Aliphatic and Aromatic Amines and Amides (JECFA, 2008b)**

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4) or 5)]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
16.102 1772	2,3,4,5,6-Pentahydroxy-N-(2-hydroxyethyl)-hexanamide		24 13	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach. Register name to be changed to (2R,3S,4S,5R)-2,3,4,5,6-Pentahydroxy-N-(2-hydroxyethyl)hexanamide.
16.103 1774	(2R)-2-Hydroxy-N-(2-hydroxyethyl)propanamide		24 10	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach. CASrn refers to the racemate. Register name or CASrn to be changed accordingly.
16.104 1775	2-[(2-Hydroxypropanoyl)amino]ethyl dihydrogen phosphate		12 5	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
16.105 1773	2-[(2,3,4,5,6-Pentahydroxyhexanoyl)amino]ethyl dihydrogen phosphate		12 3	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach. CASrn refers to the (2R,3S,4S,5R) isomer. Register name to be changed accordingly.
17.035 1771	4-Amino-butyric acid		0.12 0.1	Class I A3: Intake below threshold	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
16.090 1777	3-(3,4-Dimethoxy-phenyl)-N-[2-(3,4-dimethoxyphenyl)-ethyl]-acrylamide		1.2 1	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	Toxicity data required.	Composition of stereoisomeric mixture to be specified.

**Table 3.1: Summary of Safety Evaluation of Aliphatic and Aromatic Amines and Amides (JECFA, 2008b)**

FL-no JECFA-no	EU Register name	Structural formula	EU MSDI 1) US MSDI (µg/capita/day)	Class 2) Evaluation procedure path 3)	Outcome on the named compound [4] or 5]	EFSA conclusion on the named compound (Procedure steps, intake estimates, NOAEL, genotoxicity)	EFSA conclusion on the material of commerce
16.095 1779	N-3,7-Dimethyl-2,6-octadienyl cyclopropylcarboxamide		61 31	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach. CASrn refers to (E) isomer. Register name to be changed accordingly.
16.098 1767	N-(1-Propylbutyl)-1,3- benzodioxole-5-carboxamide		0.012 0.1	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
16.099 1768	N-(2,4-Dimethoxy-benzyl)-N'-(2- pyridin-2-yl-ethyl)-oxalamide		0.012 0.2	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
16.100 1769	N1-(2-Methoxy-4-methylbenzyl)- N2-(2-(5-methylpyridin-2- yl)ethyl)oxalamide		0.012 0.01	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
16.101 1770	N1-(2-Methoxy-4-methylbenzyl)- N2-(2-(pyridin-2- yl)ethyl)oxalamide		0.012 0.01	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach.
16.111 1776	N-[(Ethoxycarbonyl)methyl]-p- menthane-3-carboxamide		37 34	Class III B3: Intake below threshold, B4: Adequate NOAEL exists	4)	No safety concern at the estimated level of intake based on the MSDI approach.	No safety concern at the estimated level of intake based on the MSDI approach. CASrn refers to the (1R,2S,5R) isomer. Register name to be changed accordingly.

- 1) EU MSDI: Amount added to food as flavour in (kg / year) x 10E9 / (0.1 x population in Europe (= 375 x 10E6) x 0.6 x 365) = µg/capita/day.
- 2) Thresholds of concern: Class I = 1800 µg/person/day, Class II = 540 µg/person/day, Class III = 90 µg/person/day.
- 3) Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
- 4) No safety concern based on intake calculated by the MSDI approach of the named compound.
- 5) Data must be available on the substance or closely related substances to perform a safety evaluation.

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## ABBREVIATIONS

BW	Body weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CHO	Chinese hamster ovary (cells)
CoE	Council of Europe
DNA	Deoxyribonucleic acid
DTU-NFI	Danish Technical University – National Food Institute
EFSA	The European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GLP	Good Laboratory Practise
ID	Identity
IR	Infrared spectroscopy
ISS	Istituto Superiore di Sanita
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LD <sub>50</sub>	Lethal Dose, 50 %; Median lethal dose
MSDI	Maximised Survey-derived Daily Intake
mTAMDI	Modified Theoretical Added Maximum Daily Intake
NMR	Nuclear magnetic resonance
No	Number
NOAEL	No observed adverse effect level
NOEL	No observed effect level
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PCE/NCE	Polychromatic erythrocyte/normochromatic erythrocyte ratio

(Q)SAR	(Quantitative) structure-activity relationship
SCE	Sister chromatid exchange
SCF	Scientific Committee on Food
SLRL	Sex-linked recessive lethal mutations
TAMDI	Theoretical Added Maximum Daily Intake
UDS	Unscheduled DNA synthesis
US EPA	United States Environmental Protection Agency
WHO	World Health Organisation